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The first ITS phylogeny of the genus *Cantharocybe* (Agaricales, Hygrophoraceae) with a new record of *C. virosa* from Bangladesh

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Abstract

This is the first internal transcribed spacer (ITS) phylogeny of the enigmatic genus *Cantharocybe* and includes ITS sequences from two out of the three holotype collections. Two species are reported from the Americas and only a single species from Asia. Additionally, a collection of *Cantharocybe virosa* collected from tropical Bangladesh was included in this study. This species is a new record for Bangladesh, and is characterized by its tawny gray or grayish brown pileus and stipe surface, smooth ellipsoid basidiospores, elongated necked lecythiform cystidia, a trichoderm pileipellis, and abundant clamp connections. Molecular phylogenetic analysis using ITS, and combined analyses of ITS with the large subunit of nuclear ribosomal RNA (nrLSU) showed that the collection from Bangladesh is conspecific with the Indian *C. virosa*. A large, previously unknown intron was found in the ITS of *C. brunneovelutina* and *C. virosa*, while the *C. gruberi* sequence was found to be truncated where the intron would have been inserted. The intron was not identical between *Cantharocybe* species, and may be phylogenetically informative. Morphological description, color photographs and line drawings are provided for Bangladesh collection *C. virosa*. A key to the genus *Cantharocybe* is provided.

Key words

Biogeographic distribution, tropical mushroom, molecular phylogeny, taxonomy

* Md. Iqbal Hosen and Deborah Jean Lodge contributed equally to this work.

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Introduction

The genus *Cantharocybe* was introduced by Bigelow and Smith in 1973 to accommodate *Clitocybe gruberi* Smith, based on the large yellow basidiomata, oblong to subcylindrical to elongated basidiospores and the presence of lageniform to lecythiform cheilocystidia. However, other taxa in this genus do not have large-sized yellow basidiomata and oblong to elongated basidiospores. Therefore, Lodge et al. (2014) extended the generic circumscription of the genus to include taxa with large, clitocyboid, yellow, dark brown to brownish gray basidiomata with long decurrent or adnate with decurrent tooth lamellae; abundant cheilocystidia which are usually lecythiform, sometimes with a mucronate apex, with or without a rounded capitulum; smooth, inamyloid, oblong, elongate, ellipsoid to broadly ellipsoid or rarely subglobose, basidiospores; a trichoderm or cutis pileipellis; and caulocystidia similar to cheilocystidia. To date, *Cantharocybe* unites only three known species (http://www.indexfungorum.org), *C. gruberi* (Smith) Bigelow & Smith, *C. brunneovelutina* Lodge, Ovrebo & Aime and *C. virosa* (Manim. & K.B. Vrinda) T.K.A. Kumar, found in North America and Spain, Belize in Central America and India, respectively (Bigelow and Smith 1973, Justo et al. 2010, Esteves-Raventós et al. 2011, Ovrebo et al. 2011, Kumar and Manimohan 2013). Although *C. gruberi* was reported from China by Bi et al. (1993), the voucher specimen was re-identified as *Oudemansiella bii* Zhu L. Yang & Li F. Zhang (Yang and Zhang 2003). Recent molecular phylogenetic studies show that *Cantharocybe* is at the base of the hygrophoroid clade and is sister to *Ampulloclitocybe* (Pers.) Redhead, Lutzoni, Moncalvo & Vilgalys, but it is not clear if *Cantharocybe* and *Cuphophyllus* Donk (Bon) are members of Hygrophoraceae s.s. (Matheny et al. 2006, Binder et al. 2010, Lodge et al. 2014).

The phylogenetic relationships among the taxa of *Cantharocybe* are well resolved, based on partial nrLSU sequence analyses (Ovrebo et al. 2011, Kumar and Manimohan 2013). Except for *C. gruberi* (non-holotype sequences from Esteves-Raventós et al. 2011), ITS sequences for other taxa of *Cantharocybe* are unavailable. Since the ITS region is an informative genetic region for species recognition in many groups of fungi (Schoch et al. 2012), we generated ITS sequences from holotype specimens of *C. brunneovelutina* and *C. virosa*. This is the first publication with ITS sequences from all holotype collections of *Cantharocybe* except *C. gruberi*, which we used to elucidate their phylogenetic relationships.

The first author has recently collected *Cantharocybe* material from tropical Bangladesh that is morphologically similar to *C. virosa* to some extent. Careful microscopic observation of the material from Bangladesh indicates that it could be conspecific with the Indian *C. virosa*, but the nrLSU sequence analysis suggested that it could be a new species or perhaps a variety of *C. virosa*. Therefore we attempted to obtain the holotype material of *C. virosa* from TENN in order to generate additional sequences and compare with the collection from Bangladesh. Fortunately, we received cloned ITS sequence of the holotype *C. virosa* (TENN 63483) from K.W. Hughes (Tennessee, USA) that we included in our further phylogenetic studies. The goal of this study is to

elucidate the taxonomic position of the Bangladeshi collection of *Cantharocybe*, and clarify the confusion with an Indian collection of *C. virosa* using morphological and molecular evidence.

Materials and methods

Collection and deposition

Cantharocybe specimens (Iqbal568 and 693) were collected from Madhupur upazila of Bangladesh on ground near to or associated with the roots of *Cocos nucifera*, a tree of the plant family Arecaceae during the monsoon (June to August) of 2012–2013. Specimens examined are deposited in the Cryptogamic Herbarium of Kunming Institute of Botany of the Chinese Academy of Sciences (KUN), China; and in the private herbarium of Iqbal (PHI). *Cantharocybe brunneovelutina* was previously deposited at BRH and CFMR (Ovrebo et al. 2011).

Morphological studies

The morphological description of the basidiomata is based on field notes and documented by photographs. Color codes are according to Kornerup and Wanscher (1978). A small fragment of dried specimen was revived in H_2O, 5% KOH, and Congo red. The notation [n/m/p] is used in the descriptions of basidiospores measurements, which means *n* basidiospores from *m* basidiomata of *p* collections were measured; 20 basidiospores were measured from each voucher specimen. Dimension for basidiospores are given as (a–)b–c(–d), in which 'b–c' contains a minimum of 90% of the measured values and extreme values 'a' and 'd' are given in parentheses. $Q_m = Q \pm SD$: Q indicates the length/width ratio of a measured basidiospore, Q_m indicates to the average of Q basidiospores and SD is the standard deviation. For the pileipellis and stipitipellis observations radial-vertical section were made halfway of the pileus and stipe, respectively. Line drawings were done free hand.

Molecular studies

The protocol for DNA extraction followed that of Doyle and Doyle (1987). ITS1/ITS4 or ITS1/ITS5 (White et al. 1090) and LROR/LR5 (Vilgalys and Hester 1990) primer pairs were used for the amplification of the internal transcribed spacer region (ITS) and the large subunit nuclear ribosomal RNA (nrLSU), respectively. PCR amplification was carried out following the protocol of Hosen et al. (2013). PCR confirmation was confirmed on 1% agarose electrophoresis gels stained with ethidium bromide. The amplified PCR products were sent to a commercial sequencing provider company (BGI, China) for sequencing.

Three sequences (nrLSU: KF303143 and KX452406, ITS: KX452403) were generated from the Bangladeshi *Cantharocybe*. Additionally, two ITS sequences were also obtained from the type materials of *C. brunneovelutina* (BZ-1883: KX452404) and *C. virosa* (TENN-63483: KX452405). ITS sequences generated for this study were cloned in pMD18-T following manufacturer's instructions. The newly generated sequences were deposited in GenBank. An initial BLASTn search of the nrLSU sequence obtained from the Bangladeshi material against the NCBI database (http://www.ncbi.nlm.nih.gov/) gave *C. virosa* (=*Megacollybia virosa* Manim. & K.B. Vrinda), *C. brunneovelutina* and *C. gruberi* as closest hits, with maximum similarities of 96%, 96% and 95%, respectively. The closest nrLSU sequences including *Ampulloclitocybe* and *Cuphophyllus* were retrieved from GenBank and additional taxa were chosen after consulting Lodge et al. (2014) and then combined with nrLSU sequence from Bangladesh materials. Two additional datasets were constructed: ITS and ITS+nrLSU to clarify relationships between Indian and Bangladesh collections of *Cantharocybe*. All datasets were aligned with Mafft v.6.8 (Katoh et al. 2005) and manually adjusted with BioEdit v.7.0.9 (Hall 1999) using default settings. Maximum Likelihood (ML) and Bayesian Inference (BI) methods followed those in Hosen et al. (2013). *Phyllotopsis nidulans* (Pers.) Singer was served as outgroup for all dataset analyses as inferred from other phylogenetic studies (Ovrebo et al. 2011, Kumar and Manimohan 2013, Lodge et al. 2014).

Both ML and BI analyses were conducted using RAxML v.7.2.6 (Stamatakis 2006) and MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) with default settings. For ML analyses, the General Time Reversible Model of evolution with estimated gamma distribution was selected, and statistical support values were obtained using nonparametric bootstrapping (BS) with 1000 replicates. For BI analysis, the substitution model suitable for ITS and nrLSU datasets were determined using the Akaike Information Criterion (AIC) implemented in MrModeltest v.2.3 (Nylander 2004), and the models were SYM+G and GTR+I+G, respectively. Bayesian Inference analyses were conducted using the selected evolutionary model with four chains and generations set to 0.5 million for the ITS and nrLSU datasets, and four million for the combined dataset (ITS+nrLSU). Runs were terminated once the average standard deviation of split frequencies went below 0.01. Trees were sampled every 100 generations, with the first 25% of trees discarded as burn-in. Posterior probabilities (PP) were calculated using the "sump" and "sumt" commands implemented in MrBayes.

Results

Molecular results

Three datasets (ITS, nrLUS and ITS+nrLSU) are constructed and analysed separately. The ITS dataset includes 17 sequences of fungal taxa (Table 1) and consisting of 1052 nucleotide sites (gaps included) of which 417 are parsimony informative. The nrLSU

Table 1. Species of fungal taxa used in the molecular phylogenetic analyses.

Name of the species	Voucher/isolate or collection number	Origin	GenBank accession number	
			ITS, 5.8S	nrLSU
Ampulloclitocybe clavipes	AFTOL-ID 542	USA	AY789080	AY639881
Cantharocybe brunneovelutina	BZ-1883*	Belize	**KX452404**	HM588721
Cantharocybe gruberi	AH24539	Spain	JN006422	JN006420
Cantharocybe gruberi	DED6609	USA	-	AF261530
Cantharocybe gruberi	AFTOL-ID 1017	USA	DQ200927	DQ234540
Cantharocybe virosa	Iqbal-568	Bangladesh	**KX452403**	**KF303143**
Cantharocybe virosa	Iqbal-693	Bangladesh	-	**KX452406**
Cantharocybe virosa	TENN63483*	India	**KX452405**	JX101471
Cuphophyllus acutoides var. *pallidus*	CFMR TN-257	USA	KF291096	KF291097
Cuphophyllus adonis	CFMR CHIL-1	Chile	KF291035	KF291036
Cuphophyllus aff. *pratensis*	AFTOL-ID 1682	USA	DQ486683	DQ457650
Cuphophyllus aurantius	CFMR PR-6601	Puerto Rica	KF291099	KF291100
Cuphophyllus basidiosus	AFTOL-ID 1759	USA	DQ486684	DQ457651
Cuphophyllus borealis	BHS2009-104	-	HM020684	HM026552
Cuphophyllus canescens	AFTOL-ID 1800	USA	DQ486685	DQ457652
Cuphophyllus flavipes	Hattori-JP-6	Japan	KF291044	KF291045
Cuphophyllus fornicatus	Boertmann 2009/94 (CFMR)	Denmark	KF291123	KF291124
Cuphophyllus griseorufescens	PDD:27230	New Zealand	GU233328	GU233423
Phyllotopsis nidulans	HMJAU7272	China	GQ142019	GQ142039

Note: Newly generated sequences are highlighted in bold. An asterisk (*) at the isolate indicates holotype material.

dataset contains 19 sequences of the same taxa (Table 1) as well as two additional sequences of *Cantharocybe* with 934 aligned nucleotide sites (gaps included) of which 131 are parsimony informative. The combined (ITS+nrLSU) dataset includes 17 sequences of fungal taxa (Table 1) and consists of 1986 nucleotide (first 1052 for ITS and the next 934 for LSU, gaps included) sites including 541 that are parsimony informative. The aligned datasets are deposited in TreeBASE (S19556).

The ITS sequences of *C. brunneovelutina* and *C. virosa* are longer than normal because a ca. 210 bp intron is inserted about 70 bp after the ITS1 primer. This intron was not previously known as Blast searches turned up no matches, but the intron sequences were similar between *Cantharocybe* species. The GenBank ITS sequence DQ200927 of *C. gruberi* was found to be truncated on the 3' end, where the intron was likely inserted. We infer that the partial ITS sequence of *C. gruberi* deposited in GenBank as part of the Assembling the Fungal Tree of Life (AFTOL) project was truncated because the first 70 bp of the ITS are missing beginning at a point which coincides with the position of the intron insertion in *C. brunneovelutina* and *C. virosa*. Lodge et al. (2014) found that introns which were inserted within 100–150 bp of a primer

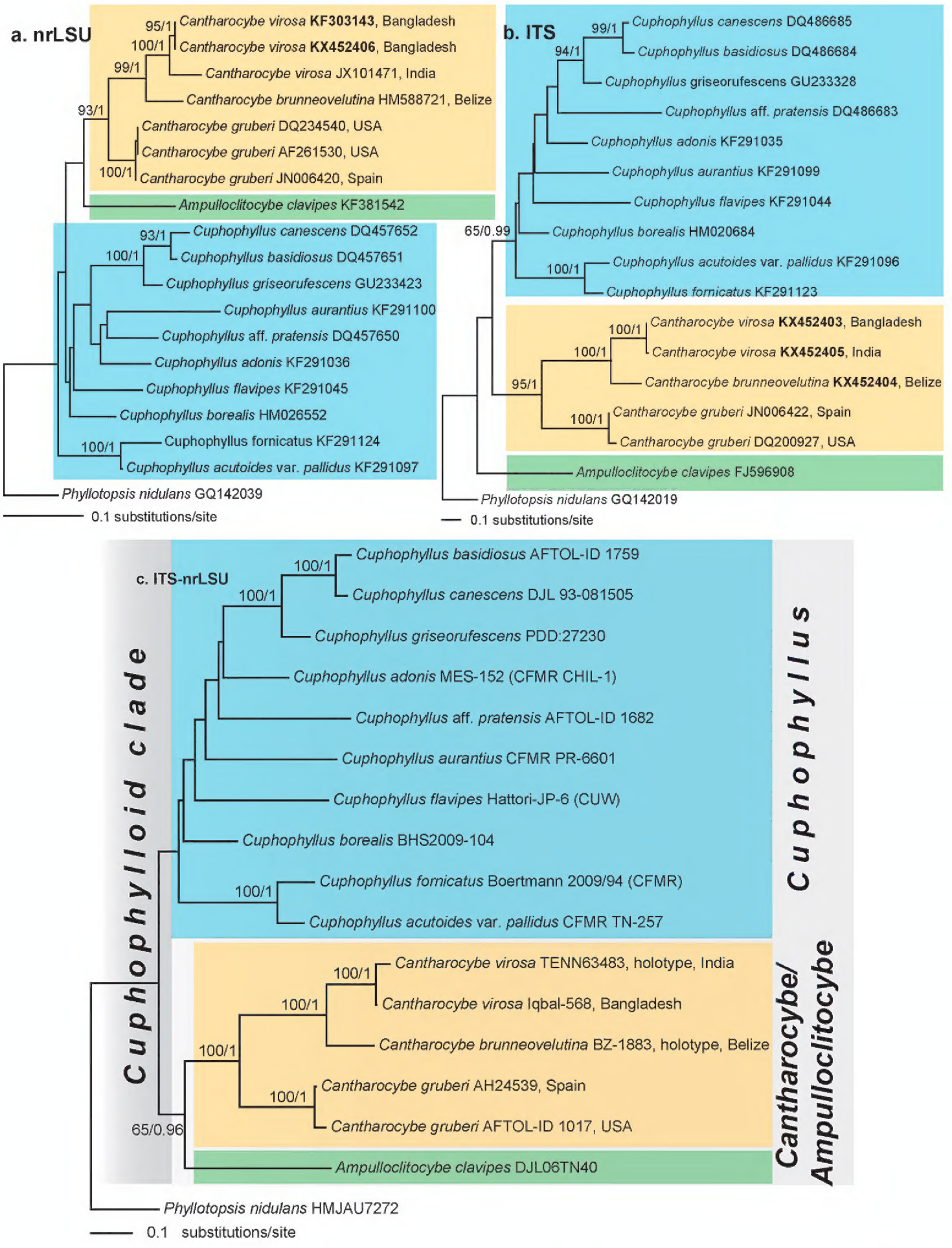

Figure 1. Phylogenetic relationships of the genus *Cantharocybe* inferred from nrLSU (**a**), ITS (**b**) and ITS+nrLSU (**c**) sequences using maximum likelihood (ML). RAxML bootstrap supports values (>50%) and Bayesian posterior probabilities (>0.95) are indicated on the branches at nodes (ML/PP). Newly generated sequences are highlighted in bold. GenBank accession or voucher numbers are provided after the species name.

disrupted replication unless the DNA was cloned using a vector such as the method we used to obtain our ITS sequences. As there is no notation in GenBank that the AFTOL ITS sequence of *C. gruberi* was cloned, we infer that forward reads were disrupted by the same intron that was found in *C. brunneovelutina* and *C. virosa*. Thus, it is likely that Matheny and Hibbett's AFTOL program only obtained back-reads from the 5' end using the ITS4 primer. The authors of the *C. gruberi* DQ200927 sequence may or may not have obtained a partial read of an intron, but if so, it would have been of diminishing quality with distance from the ITS4 primer, it would not have matched any known ITS sequences, and it would have been impossible to correct or corroborate without a forward read from the 3' end. We therefore infer that if a partial read of an intron was obtained by Matheny and Hibbett for *C. gruberi*, that it was trimmed from the GenBank submission because it did not match ITS1 sequences and it could not be corrected or corroborated. Blast searches of GenBank using the ITS sequence of *C. virosa*, after removing the intron, retrieved *C. gruberi* sequences with highest similarity.

Tree topologies obtained from both ML and BI methods of phylogenetic analyses are congruent, the ML trees are shown in Fig. 1. The Bangladesh sample of *Cantharocybe* clusters in a strongly supported clade with the Indian sample of *C. virosa* in all three datasets, indicating that they are conspecific (Fig. 1).

Taxonomy

***Cantharocybe virosa* (Manim. & K.B. Vrinda) T.K.A. Kumar**
Figs 2–3

Cantharocybe virosa (Manim. & K.B. Vrinda) T.K.A. Kumar, Mycotaxon 124: 235 (2013).
≡ *Megacollybia virosa* Manim. & K.B. Vrinda, Mycotaxon 111: 364 (2010).

Description. Basidiomata medium-sized to large. Pileus 50–80 mm diam., convex at first then applanate, sometimes uplifted with cracked margin, tawny gray, dark brown (6E4–5) to grayish brown (5E3–5E4, 6E3–6F4), dry, pruinose or with fine appressed scales under lens, margin without striation. Hymenophore lamellate; lamellae adnate to decurrent, subdistant to crowded, white to pallid white (5A1, 6A1); lamellulae numerous, concolorous with lamellae. Stipe 50–80 × 10–15 mm, central, slightly curved, cylindrical, gradually thickening towards the base, at the apex ribbed by the subdeccurent lines of the hymenophore, upper half pale gray or brownish gray (5D2) to grayish brown (5E3) pruina or squamules and the remaining half nearly concolorous with the pileus, with cottony mycelium at the base, interior solid, milky white to white. Context 12 mm thick in the center of the pileus, milky white to white (6A1), firm, solid, unchanging when cut or bruised.

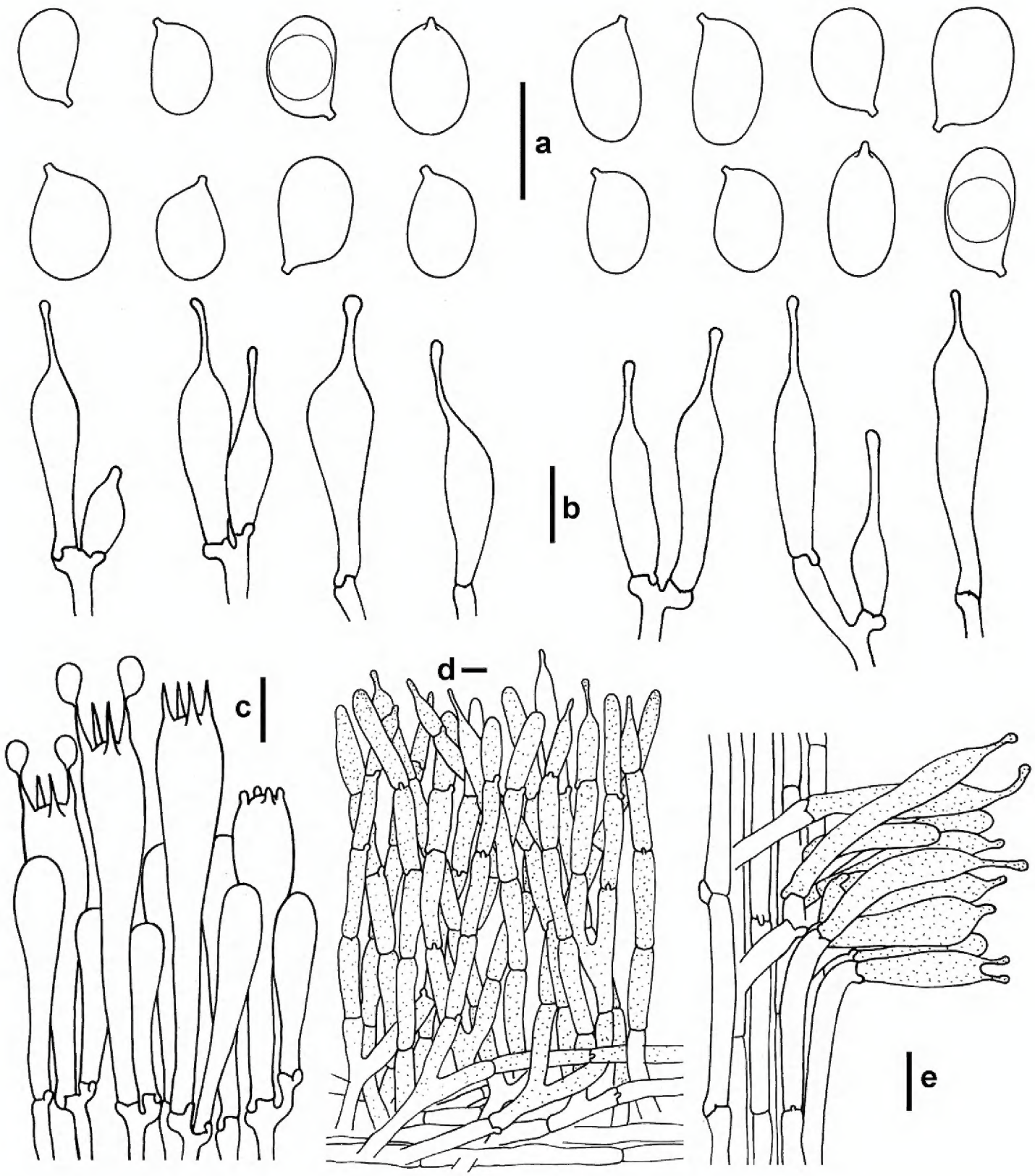

Figure 2. Microscopic features of *Cantharocybe virosa* (HKAS 79012, Iqbal 568). **a** Basidiospores **b** Cheilocystidia **c** Basidia at different stages of development **d** Trichoderm pileipellis **e** Surface of stipe in longitudinal section showing caulocystidia. Scale bars: 10 μm.

Basidiospores [40/2/2] (8–) 8.5–10(–11.5) × 5–6.2(–7.0) μm, [Q = 1.54–1.62, Q_m = 1.58 ± 0.12] ellipsoid to broadly ellipsoid, hyaline, thin-walled, smooth, inamyloid, apiculus conspicuous. Basidia 45–60 × 8–11 μm, clavate, hyaline, thin-walled, 4-spored, sterigmata up to 8 μm long; basal septum usually clamped. Cheilocystidia 25–45 × 5–9 μm, abundant, lecythiform to lageniform, sometimes with a mucronate apex, basal portion usually clavate, the upper portion extending into an elongated neck up to 15 μm long with or without a rounded capitulum; basal septa often clamped.

Figure 3. Basidiomata of *Cantharocybe virosa*. **a, c** Adnate-decurrent lamellae and pileus-stipe surface **b, d** Longitudinal section illustrating unchanged solid context **a, b** from HKAS 79012, Iqbal 568 **c, d** from Iqbal 693. Scale bars: 2 cm.

Pleurocystidia absent. Lamellar trama parallel to sub-regular composed of branching filamentous hyphae 4–10 µm wide, hyaline to pale yellow, thin-walled. Pileipellis a trichoderm, slightly interwoven, composed of 6–10 µm wide hyphae with often pale brown vacuolar to plasmatic pigments; terminal cells 20–65 × 6–10 µm, usually cylindrical to somewhat narrowly clavate, sometimes mucronate; pileocystidia with or without extending neck, occasionally with one or two short rounded capitula, elongated neck up to 15 µm long, clamp connection frequently present at septa. Stipitipellis composed of vertically arranged, branching, 7–10 µm wide filamentous hyphae, outer surface more or less covered with cylindrical to narrowly clavate cells (35–100 × 6–11 µm) with or without a rounded capitulum, mostly similar to cheilocystidia but sometimes double necked with an extending capitulum head, pale brown vacuolar to plasmatic pigments. Clamp connections common at septa.

Habitat. Solitary or in clusters, associated with roots of *Cocos nucifera* (collection Iqbal 568) or along the roadside on ground (collection Iqbal 693) near *C. nucifera*.

Distribution. Known from tropical South Asia, Bangladesh and India.

Specimens examined. Bangladesh, Dhaka division: Tangail, Madhupur, Bangladesh Agricultural Development Corporation (BADC) campus, 24°37'35"N,

90°03'33"E, 05 Aug 2012, 20–25 m, Iqbal 568 (HKAS 79012, PHI-12); same location, 18 Jun 2013, Iqbal 693 (PHI-13). Belize, Orange walk district: La Milpa Field State, La Milpa Archaeological Site, 17°50'30"N, 89°1'0"W, 100 m (CFMR), 25 Oct 2002, DJL-BZ-85 (BZ-1883).

Discussion

Morphology and phylogenetic relationships of *Cantharocybe*

The Bangladeshi *C. virosa* is characterized by its gray to grayish brown basidiomata, moderately crowded lamellae, fine squamules on stipe surface formed from clusters of lecythiform caulocystidia, ellipsoid to broadly ellipsoid basidiospores, and a trichoderm pileipellis.

Based on molecular analyses, *C. virosa*, a species recently described from India is conspecific with the Bangladeshi collection (Fig. 1). However, the Indian *C. virosa* has a pale grayish brown pileus, long cheilocystidia which can be up to 63 µm with a long neck up to 35 µm, a cutis pileipellis or occasionally disrupted with trichodermal patches (Kumar and Manimohan 2013). In comparison, the Bangladeshi collection has a grayish brown to dark brown pileus, cheilocystida with short neck up to 15 µm long, and clearly defined trichoderm pileipellis. Geographically, *C. virosa* is distributed in the Kerala state (South-West region) of India, while the new collection was collected from Tangail district of the Dhaka division of Bangladesh. Moreover, the nrLSU sequence obtained from the Bangladeshi collection does not perfectly match (96%) with the holotype *C. virosa* retrieved from GenBank, and the genetic distance between them is 0.96% (8 bases differences and 23 deletions in the Indian collection) of 831 nucleotide sites. These morphological variations, geographic distance and nrLSU sequence inferred suggest that they may have diverged recently from each other due to its allopatric speciation.

Surprisingly, when we blasted the newly generated ITS sequences from the holotypes of *C. virosa* (TENN 63483) and *C. brunneovelutina* (BZ-1883) individually against the NCBI database, we did not find *C. gruberi* as the closest sister species among the first 100 matched species, even 80–88% matched only with some taxa of *Tricholoma*, *Lepista*, *Macrolepiota*, *Lepiota*, etc. The ITS sequence from the newly collected material from Bangladesh also gave a similar result. These results are caused by the presence of a large intron in *C. brunneovelutina* and *C. virosa*, and the truncated sequence of *C. gruberi* deposited in GenBank (missing the intron and 3' end). Subsequently, we retrieved the closest ITS sequences of those taxa including *C. gruberi* from GenBank and reconstructed an ITS phylogenetic tree where all *Cantharocybe* taxa were nested together within the same clade with strong BS value (99% ML BS, 1.0 PP) and apart from *Tricholoma* and *Lepista* clades (data not shown). In the ITS sequence analyses, the Indian entity *C. virosa* showed only 5 base pair difference with 4 deletions out of 742 nucleotides (genetic distance 0.68%) from the Bangladeshi material. Fur-

ther extended combined dataset (ITS+nrLSU) also showed little divergence (genetic distance 0.83%) between them (Fig. 1c). This small variation in ITS sequences, with negligible differences in the color of the basidioma, size of basidia and cheilocystidia which were possibly due to environmental variations, do not warrant a new variety or species, suggesting that both south Asian entities are conspecific. Furthermore, both collections were the same ecology and associated with the roots of *C. nucifera* in a tropical region. Neither coconut trees nor palms in general have been shown to associate with ectomycorrhizal fungi. Halbwachs et al. (2013) however, found hyphae of a *Cuphophyllus* species (a genus near *Cantharocybe*) and several species of *Hygrocybe* as endophytes in plant roots including those of a monocot (*Plantago major*) so another type of root symbiosis with *C. nucifera* is possible. The considerable variation in the nrLSU sequence (96% match, 8 bases differences with 23 deletion) of the Indian *C. virosa* may be explained by the fact that the nrLSU sequence obtained from that collection was not clean, showing evidence of a contaminating sequence or minor indel (K.W. Hughes, pers. comm.).

Cantharocybe brunneovelutina and *C. gruberi* can also be separated from *C. virosa* morphologically. *Cantharocybe brunneovelutina* differs from *C. virosa* by its velutinous basidioma, unusual cheilocystidia with multiple prong-like appendages at the apex resembling a basidia-like structure, and a trichoderm pileipellis (Ovrebo et al. 2011). The type species of this genus, *C. gruberi*, has a pale yellow to lemon yellow pileus and narrowly elliptical to oblong basidiospores measuring 11–16(–17) × (4.5–) 6–7.5 µm (Bigelow and Smith 1973).

Molecular phylogenetic analyses indicated that the genus *Cantharocybe* is monophyletic, with strong bootstrap values (Fig. 1). Likewise, Ovrebo et al. (2011) and Lodge et al. (2014) showed that the monophyletic clade of *Cantharocybe* has strong BS value comprising *C. gruberi* and *C. brunneovelutina* using single locus or multi-locus sequence analyses. Although recent phylogenetic studies (Ovrebo et al. 2011, Kumar and Manimohan 2013, Lodge et al. 2014) showed the monophyly of *Cantharocybe*, the sister relationship with other genera remains unclear. *Cantharocybe* nests at the base of the hygrophoroid clade together with *Ampulloclitocybe* and *Cuphophyllus* (Binder et al. 2010, Matheny et al. 2006, Ovrebo et al. 2011, Lodge et al. 2014), but their relationships were not confidently resolved. In our combined dataset (ITS+nrLSU) analysis, *Ampulloclitocybe* is only weakly supported (65% ML BS, PP = 0.96) as sister to the *Cuphophylloid* clade (Fig. 1c). This is accordance with the recent phylogenetic study of Lodge et al. (2014).

Distribution and ecology of *Cantharocybe*

Cantharocybe is an uncommon genus that only consists of three species. *Cantharocybe gruberi* has wide distribution from America (New Mexico, western North America and British Columbia) to Europe (Spain). *Cantharocybe brunneovelutina* is reported from tropical Central America (Belize) whereas *C. virosa* is from tropical South Asia

(Bangladesh and India). Based on the branching order with strong bootstrap support at all nodes in our phylogeny in which *C. gruberi* is basal, we infer that the genus *Cantharocybe* may have originated in America or Europe and then migrated independently to Central America and South Asia.

The south Asian species usually occurs with the roots of *Cocos nucifera* (Manimohan et al. 2010, Kumar and Manimohan 2013, this study as well), the North American and European collections were found on needle beds or ground under conifers and *Pinus nigra*, respectively (Bigelow and Smith 1973, Esteves-Raventós et al. 2011), and the Central American species was found in humus around dead palm trees (Ovrebo et al. 2011). However, their symbiotic association with trees is still unknown. To facilitate identification of *Cantharocybe* taxa worldwide, a key to the species is given bellow.

Key to the taxa of Cantharocybe

1 Basidiomata medium (up to 75 mm broad), with dark brown pileus, cheilocystidia resembling basidia with apical appendages, basidiospores elliptical to oblong or subcylindrical, Belize (Central America, tropical)..... ***C. brunneovelutina***

– Basidiomata medium to large (45–200 mm broad), yellow or pale grayish brown to grayish brown, basidiospores oblong to cylindrical or ellipsoid to broadly ellipsoid or rarely subglobose, cheilocystidia lecythiform..... **2**

2 Basidiomata large (up to 200 mm broad), yellow, basidiospores 11–16(–17.5) × (4.5–) 6–7.5 µm, elliptical, oblong to cylindrical, western North America (temperate zone) and Spain (Europe)..... ***C. gruberi***

– Basidiomata medium (up to 100 mm broad), pale grayish brown to tawny gray to grayish brown, basidiospores 6.5–11(–12) × 5.5–6.5(–7) µm, ellipsoid to broadly ellipsoid or rarely subglobose, Bangladesh and India (South Asia, tropical)..... ***C. virosa***

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References

Bi Z, Zheng G, Li TH (1993) The macrofungus flora of China's Guangdong Province. The Chinese University Press, Hong Kong, 734 pp.

Bigelow HE, Smith AH (1973) *Cantharocybe*, a new genus of Agaricales. Mycologia 65: 485–488. doi: 10.2307/3758121

Binder M, Larsson KH, Matheny PB, Hibbett DS (2010) Amylocorticiales ord. nov. and Jaapiales ord. nov.: early diverging clades of Agaricomycetidae dominated by corticioid forms. Mycologia 102: 865–880. doi: 10.3852/09-288

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Esteves-Raventós F, Alvarado P, Reyes JD, Manjón JL (2011) Nuevos datos sobre la identidad de Pleurotus dryinus var. luteosaturatus (Agaricales) sobre la base de estudios morfológicos y moleculares. Boletín de la Sociedad Micológica de Madrid 35: 77–83.

Halbwachs H, Dentinger BTM, Detheridge AP, Karasch P, Grifith GW (2013) Hyphae of waxcap fungi colonise plant roots. Fungal Ecology 6: 487–492. doi: 10.1016/j.funeco.2013.08.003

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analyses program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hosen MI, Feng B, Wu G, Zhu XT, Li YC, Yang ZL (2013) *Borofutus*, a new genus of Boletaceae from tropical Asia: morphology, taxonomy and phylogeny. Fungal Diversity 58: 215–226. doi: 10.1007/s13225-012-0211-8

Justo A, Vizzini A, Minnis AM, Menolli JrN, Capelari M, Iguez OR, Malysheva E, Contu M, Ghignone S, Hibbett DS (2010) Phylogeny of the Pluteaceae (Agaricales, Basidiomycota): taxonomy and character evolution. Fungal Biology 115: 1–20. doi: 10.1016/j.funbio.2010.09.012

Katoh K, Kuma K, Toh H, Miyata T (2005) MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Research 33: 511–518. doi: 10.1093/nar/gki198

Kornerup A, Wanscher JH (1978) Methuen handbook of colour (3rd edn). Eyre Methuen, London, 252 pp.

Kumar TKA, Manimohan P (2013) Molecular phylogeny reveals *Megacollybia virosa* is a *Cantharocybe*. Mycotaxon 124: 231–238. doi: 10.5248/124.231

Lodge DJ, Padamsee M, Matheny PB, Aime MC, Cantrell SA, Boertmann D, Kovalenko A, Vizzini A, Dentinger BTM, Kirk PM, Ainsworth AM, Moncalvo JM, Vilgalys R, Larsson E, Lücking R, Griffith GW, Smith ME, Norvell LL, Desjardin DE, Redhead SA, Ovrebo CL, Lickey EB, Ercole E, Hughes KW, Courtecuisse R, Young A, Binder M, Minnis AM, Lindner DL, Ortiz-Santana B, Haight J, Læssøe T, Baroni TJ, Geml J, Hattori T (2014) Molecular phylogeny, morphology, pigment chemistry and ecology in Hygrophoraceae (Agaricales). Fungal Diversity 64: 1–99. doi: 10.1007/s13225-013-0259-0

Manimohan P, Kumar TKA, Vrinda KB, Pradeep CK (2010) *Megacollybia virosa*, a new species with toxic basidiomata from India. Mycotaxon 111: 363–368. doi: 10.5248/111.363

Matheny PB, Curtis JM, Hofstetter V, Aime MC, Moncalvo JM, Ge ZW, Yang ZL, Slot JC, Ammirati JF, Baroni TJ, Bougher NL, Hughes KW, Lodge DJ, Kerrigan RW, Seidl MT, Aanen DK, DeNitis M, Daniele GM, Desjardin DE, Kropp BR, Norvell LL, Parker A, Vellinga EC, Vilgalys R, Hibbett DS (2006) Major clades of Agaricales: a multilocus phylogenetic overview. Mycologia 98: 982–995. doi: 10.3852/mycologia.98.6.982

Nylander JAA (2004) MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Ovrebo CL, Lodge DJ, Aime MC (2011) A new *Cantharocybe* from Belize with notes on the type of *Cantharocybe gruberi*. Mycologia 103: 1102–1109. doi: 10.3852/10-360

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572–1574. doi: 10.1093/bioinformatics/btg180

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W (2012) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences, USA 109: 6241–6246. doi: 10.1073/pnas.1117018109

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. doi: 10.1093/bioinformatics/btl446

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, 315–322. doi: 10.1016/b978-0-12-372180-8.50042-1

Yang ZL, Zhang LF (2003) Type studies on *Clitocybe macrospora*, and *Xerula furfuracea* var. *bispora*. Mycotaxon 88: 447–454.